

## FIELD OF THE INVENTION

## BACKGROUND OF THE INVENTION

10 a) Spray dried products, wherein a liquid enzyme-containing solution is atomised in a spray drying tower to form small droplets which during their way down the drying tower dry to form an enzyme-containing particulate material. Very small particles can be produced this way (Michael S. Showell  
15 (editor); *Powdered detergents*; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker).

b) Layered products, wherein the enzyme is coated as a layer around a pre-formed inert core particle, wherein an enzyme-containing solution is atomised, typically in a fluid bed apparatus wherein the pre-formed core particles are fluidised, and the enzyme-containing solution adheres to the core particles and dries up to leave a layer of dry enzyme on the surface of the core particle. Particles of a desired size can be obtained this way if a useful core particle of the desired size can be found. This type of product is described in e.g. WO 97/23606

c) Absorbed core particles, wherein rather than coating the enzyme as a layer around the core, the enzyme is absorbed onto and/or into the surface of the core. Such a process is described in WO 97/39116.

d) Extrusion or pelletized products, wherein an enzyme-containing paste is pressed to pellets or under pressure is extruded through a small opening and cut into particles which are subsequently dried. Such particles usually have a considerable size because of the material in which the extrusion opening is made (usually a plate with bore holes) sets a limit on the allowable pressure drop over the extrusion opening. Also, very high extrusion pressures when using a small opening increase heat generation in the enzyme paste, which is harmful to the enzyme. (Michael S. Showell (editor); *Powdered detergents*; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker)

e) Prilled products or, wherein an enzyme powder is suspended in molten wax and the suspension is sprayed, e.g. through a rotating disk atomiser, into a cooling chamber where the droplets quickly solidify (Michael S. Showell (editor); *Powdered detergents*; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker). The product obtained is one wherein the enzyme is uniformly distributed throughout an inert material instead of being concentrated on its surface. Also US 4,016,040 and US 4,713,245 are documents relating to this technique

f) Mixer granulation products, wherein an enzyme-containing liquid is added to a dry powder composition of conventional granulating components. The liquid and the powder in a suitable proportion are mixed and as the moisture of the liquid is absorbed in the dry powder, the components of the dry powder will start to adhere and agglomerate and particles will build up, forming granulates comprising the enzyme. Such a process is described in US 4,106,991 (NOVO NORDISK) and related documents EP 170360 B1 (NOVO NORDISK), EP 304332 B1 (NOVO NORDISK), EP 304331 (NOVO NORDISK), WO 90/09440 (NOVO NORDISK) and WO 90/09428 (NOVO NORDISK). In a particular product of this

process wherein various high-shear mixers can be used as granulators, granulates consisting of the enzyme, fillers and binders etc. are mixed with cellulose fibres to reinforce the particles to give the so-called T-granulate. Reinforced  
5 particles, being more robust, release less enzymatic dust (*vide infra*).

#### **DRAWINGS**

No drawings

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#### **SUMMARY OF THE INVENTION**

The present invention relates to an enzyme-containing granule comprising a core unit and a shell unit, wherein the core unit comprises the enzyme and is enclosed in a shell unit  
15 which is substantially enzyme-free, the ratio between the diameter of the granule and the diameter of the core unit being at least 1.1.

In a second aspect, the invention relates to a process for preparing enzyme core units and finished enzyme granules,  
20 comprising the enzyme core unit and the shell unit. The invention further relates to compositions comprising the enzyme granule such as foodstuff/baking/flour/dough compositions or detergent composition and the use of such compositions in application.

25 In further aspects the invention relates to specific processes for preparing enzyme containing core units.

#### **DETAILED DESCRIPTION OF THE INVENTION**

##### **30 Definitions**

The phrase "ratio between the diameter of the granule and the diameter of the core unit and" (hereinafter abbreviated  $D_G/D_C$ ) as used herein is to be understood as the diameter of the granule comprising a core unit and a shell unit divided by the

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diameter of the core unit only. If for example a core unit having a diameter of 100  $\mu\text{m}$  is coated with a coating layer 200  $\mu\text{m}$  thick, the granule would have a diameter of  $(200+100+200)=500 \mu\text{m}$  and  $D_G/D_C$  is  $500 \mu\text{m}/100 \mu\text{m} = 5$ .

5 The term "activity" when used in reference to an enzyme preparation or with reference to an enzyme granule or an enzyme core is a relative measure of the ability of the enzyme in the preparation, granule or core to react with a standard substrate  
 10 at fixed standard conditions. Activity is measured in units which is defined as  $\mu\text{moles}$  of substrate reacted per minute per gram of the measured sample at fixed standard conditions (herein after "a standard assay"). The activity is also a measure of the amount of active enzyme protein. An enzyme has a  
 15 specific activity which is the activity of the pure enzyme protein in the standard assay. The specific activity is also measured in units which is defined as  $\mu\text{moles}$  of substrate reacted per minute per gram of pure enzyme at fixed standard conditions. When the specific activity of an enzyme is known  
 20 the amount of pure enzyme protein in a sample can be calculated. If a 1 gram sample of a pure enzyme react with 100  $\mu\text{moles}$  of a substrate per minute in a standard assay, the specific activity of the enzyme is 100 Units per gram pure enzyme. If a 1 gram sample of unknown enzyme activity reacts  
 25 with 50  $\mu\text{moles}$  of a substrate per minute in the standard assay, the activity of the sample is 50 Units per gram and there is 0.5 g of pure enzyme protein in the sample.

It is to be understood that the term "size" of particles or  
 30 granulates covers the diameter of a particle measured in the longest dimension of the particle or granulate. Also, the mean size of granules is to be understood as the mean diameter of granules manufactured by the process of the invention measured in the longest dimension of the particles. The term "particle  
 35 size distribution" is meant to be understood the range of sizes

of granules resulting from a particular process; the spectrum or gradient distribution of granules with regards to their diameter.

5 The particle size distribution (PSD) can be expressed in terms of the mass mean diameter of the individual particles. A mean mass diameter of D50 is the diameter at which 50% of the granules, by mass, have a smaller diameter, while 50% by mass have a larger diameter. The values D10 and D90 are the  
10 diameters at which 10% and 90%, respectively, of the granules, by mass, have a smaller diameter than the value in question. The "span" indicates the breadth of the PSD and is expressed as:

$$(D90 - D10) / D50.$$

15 For purposes of the present invention, the particle size distribution is normally as narrow as possible. The span of a granulate product according to the invention is therefore typically not more than about 2.5, preferably not more than about 2.0, more preferably not more than about 1.5, and most  
20 preferably not more than about 1.0 such as between 0.1 to 0.9.

The terms "particle" and "granulate" or "granule" are to be understood as predominantly spherical or near spherical structures of a macromolecular size.

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The term "substantially enzyme free" as used herein about a shell unit means that there less than 5 mg of enzyme per gram shell.

30 The term "Rayleigh Atomizer" is to be understood as an atomizer capable of producing droplets of liquid having a low SPAN value (usually SPAN values below 1.5 can be obtained such as between 0.9-1.3), said atomizer characterized by comprising a spraying member and a surface member comprising at least one bore hole.  
35 In a preferred embodiment the Rayleigh Atomizer is a rotating

atomizing device wherein a liquid is atomized by distributing the liquid onto the inner surface of a rotating hollow cylinder comprising bore holes, the liquid forming droplets by passing the cylinder wall through the bore holes. Such an atomizer is described in WO 94/21383 claims 9-30 and figures 1-18 and methods for atomizing in claims 1-8 all incorporated herein by reference. The principles and mechanics of Rayleigh atomization are known to the art.

#### 10 **The Granule**

In enzyme granules of the invention, enzymatic activity is concentrated into a central core unit surrounded a shell unit or coating which is substantially enzyme free. The core unit is smaller than core units known to the art and the shell unit is thicker than shell units known to the art and in order to provide enzyme granules having a total activity useful in established applications of enzyme granules the enzyme activity in the core unit is considerably augmented. The smaller enzyme cores of the invention, which can be prepared having a very narrow particle size distribution provides new and flexible preparation ways of controlling the activity by independently varying the size of the enzyme core and the thickness of the surrounding shell. Moreover, the enzyme granules of the invention have environmentally advantageous properties such as low dust and odour levels, reduced contents of granulate additive and improved storage stability of the enzyme. The products may have a natural white colour circumventing the need for expensive and environmentally antagonistic pigments such as titanium dioxide pigments in additional coatings.

The granule of the invention is characterised by having a structure wherein  $D_g/D_c$  is at least 1.1, which means that the thickness of the shell unit is at least 5% of the core unit diameter. The more the enzyme activity can be concentrated in the core unit, the smaller the core unit can be made, and the thicker the shell unit can be made, when preparing a granule of

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## The Enzyme Core Unit

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The intention is to concentrate the enzyme content to a small central fraction of the overall granulate. This small fraction, herein termed the enzyme core unit, although intended to be small in size, must at least be large enough to prevent its agglomeration with other enzyme core units during the granulation process by shell coating components. To prevent agglomeration of the enzyme core unit during further processing however, the size of the enzyme core unit is preferably greater than 50  $\mu\text{m}$ , such as greater than 100  $\mu\text{m}$ . This may correspond to an enzyme core unit of at least 1% by weight of the total mass, such as at least 2%, such as at least 5% or 10% of the total mass. In a preferred embodiment the core constitutes between 1 to 5% w/w of the granule.

An integral feature of the present invention is that enzyme activity is limited solely to the core unit. No other moiety or component of the granule as defined by this invention is intended to contain enzymes. As is known by the person skilled in the art however, the enzyme may be dispersed or diffused elsewhere during the use of the final granulate.

The physical state of the enzyme core can be that of a  
30 solid, liquid, or gel.

Preferable embodiments of the invention comprise a solid enzyme core unit. In one embodiment of the invention, the enzyme core unit is solid when encased in its shell unit. Thereafter, the enzyme granule can be heated above the melting



point of the binders or other components of the enzyme core so as to cause these components to diffuse into the inner parts of the shell unit resulting in an increase porosity of the enzyme core. This will in turn increase the solubility of the core unit.

### Enzymes

The enzyme in the context of the present invention may be any enzyme or combination of different enzymes. Accordingly, when reference is made to "an enzyme" this will in general be understood to include both a single enzyme and a combination of more than one enzyme.

It is to be understood that enzyme variants (produced, for example, by recombinant techniques) are included within the meaning of the term "enzyme". Examples of such enzyme variants are disclosed, e.g., in EP 251,446 (Genencor), WO 91/00345 (Novo Nordisk), EP 525,610 (Solvay) and WO 94/02618 (Gist-Brocades NV). The enzyme classification employed in the present specification and claims is in accordance with *Recommendations (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology*, Academic Press, Inc., 1992.

Accordingly the types of enzymes which may appropriately be incorporated in granules of the invention include oxidoreductases (EC 1.-.-.-), transferases (EC 2.-.-.-), hydrolases (EC 3.-.-.-), lyases (EC 4.-.-.-), isomerases (EC 5.-.-.-) and ligases (EC 6.-.-.-).

Preferred oxidoreductases in the context of the invention are peroxidases (EC 1.11.1), laccases (EC 1.10.3.2) and glucose oxidases (EC 1.1.3.4)], while preferred transferases are transferases in any of the following sub-classes:

- a) Transferases transferring one-carbon groups (EC 2.1);
- b) Transferases transferring aldehyde or ketone residues (EC 2.2); acyltransferases (EC 2.3);

A most preferred type of transferase in the context of the invention is a transglutaminase (protein-glutamine  $\gamma$ -glutamyltransferase; EC 2.3.2.13).

10 Further examples of suitable transglutaminases are described in  
WO 96/06931 (Novo Nordisk A/S).

Preferred hydrolases in the context of the invention are: Carboxylic ester hydrolases (EC 3.1.1.-) such as lipases (EC 3.1.1.3); phytases (EC 3.1.3.-), e.g. 3-phytases (EC 3.1.3.8) and 6-phytases (EC 3.1.3.26); glycosidases (EC 3.2, which fall within a group denoted herein as "carbohydrases"), such as  $\alpha$ -amylases (EC 3.2.1.1); peptidases (EC 3.4, also known as proteases); and other carbonyl hydrolases].

In the present context, the term "carbohydrase" is used to denote not only enzymes capable of breaking down carbohydrate chains (e.g. starches) of especially five- and six-membered ring structures (i.e. glycosidases, EC 3.2), but also enzymes capable of isomerizing carbohydrates, e.g. six-membered ring structures such as D-glucose to five-membered ring structures such as D-fructose.

Carbohydrases of relevance include the following (EC numbers in  
30 parentheses):

$\alpha$ -amylases (3.2.1.1),  $\beta$ -amylases (3.2.1.2), glucan 1,4- $\alpha$ -glucosidases (3.2.1.3), cellulases (3.2.1.4), endo-1,3(4)- $\beta$ -glucanases (3.2.1.6), endo-1,4- $\beta$ -xylanases (3.2.1.8), dextranases (3.2.1.11), chitinases (3.2.1.14),

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1.-.-.-) include Gluzyme™

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Alpha-Gal™, Bio-Feed™ Alpha, Bio-Feed™ Beta, Bio-Feed™ Plus,

15 weight .

However some enzymes have a very high specific activity so that less enzyme protein by weight is required to maintain a high activity of the core unit. Accordingly for e.g. a protease a preferred core activity is at least 60 KNPU per gram core, 20 more preferably at least 100 KNPU, more preferably at least 200 KNPU or most preferably at least 250 KNPU per gram core. The unit for protease activity used herein is Kilo Novo Protease Units per gram of sample (KNPU/g). The enzyme activity is determined in a standard assay by measuring for a given amount 25 of core the formation rate ( $\mu\text{mol/minute}$ ) of free amino groups liberated from digestion of di-methyl-casein (DMC) in solution by the enzyme. The formation rate is monitored by recording the linear development of absorbance at 420 nm of the simultaneous reaction between the formed free amino groups and added 2,4,6- 30 tri-nitro-benzene-sulfonic acid (TNBS). The digestion of DMC and the colour reaction is carried out at 50°C in a pH 8.3 boric acid buffer with a 9 min. reaction time followed by a 3 min. measuring time. A folder AF 220/1 is available upon



5 than 20% w/w of a known enzyme using known methods.

is homogeneously distributed or dispersed within the core unit.

10 will for example typically be in the range of 1-20 KNPu/g,

15 purpose of the granulate. The term "enzyme" and some preferred

25 different enzymes and may be optionally coated. An alternative

layered enzyme core unit. Granules comprising of a structured

co-granules have protease and amylase activities, but other combinations, such as protease-lipase-carbohydrase and many other combinations of 2 or 3 activities and/or enzymes are also possible. Co-granules can be as layered structures or as clustered-particle structures.

#### Excipients

The enzyme core unit can comprise excipients or additives, which may serve a specialised function in the core unit.  
10 Excipients may be compounds conventionally used in the art, and may be selected from the non limiting group of:

- Enzyme stabilising agents. Enzyme stabilising or protective agents such as conventionally used in the field of granulation may be elements of the enzyme-containing unit. Stabilising or protective agents may fall into several categories: alkaline or neutral materials, reducing agents, antioxidants and/or salts of first transition series metal ions. Each of these may be used in conjunction with other protective agents of the same or different categories. Examples of alkaline protective agents are alkali metal silicates, carbonates or bicarbonates which provide a chemical scavenging effect by actively neutralising e.g. oxidants. Examples of reducing protective agents are salts of sulfite, thiosulfite or thiosulfate, while examples of antioxidants are methionine, butylated hydroxytoluene (BHT) or butylated hydroxyanisol (BHA). Most preferred agents are salts of thiosulfates, e.g. sodium thiosulfate or methionine. Also enzyme stabilizers may be borates, borax, formates, di- and tricarboxylic acids and reversible enzyme inhibitors such as organic compounds with sulfhydryl groups or alkylated or arylated boric acids. Examples of boron based stabilizer may be found in WO 96/21716, whereas a preferred boron based stabilizer is 4-Formyl-Phenyl-

Boronic Acid or derivatives thereof described in WO 96/41859 both disclosed incorporated herein by reference. Still other examples of useful enzyme stabilizers are gelatine, casein, Poly vinyl pyrrolidone (PVP) and powder of skimmed milk. Enzyme stabilising agents may constitute be 0.01-10% w/w of the core unit, preferably 0.1-5%, e.g. 0.5-2.5% w/w of the core unit.

- Solubilising agents. The solubility of the enzyme core unit is especially critical in cases where the unit is a component of detergent formulation. As is known by the person skilled in the art, many agents, through a variety of methods, serve to increase the solubility of formulations, and typical agents known to the art can be found in national *Pharmacopeia*'s. Thus, the enzyme core unit may optionally comprise any agent that serves to enhance the solubility of the enzyme core unit. These agents usually cause the formulation to swell upon contact with water, or to disintegrate, rupture, burst or break open.
- Inorganics, such as water soluble and/or insoluble inorganic salts such as finely ground alkali sulphate, alkali carbonate and/or alkali chloride, clays such as kaolin (e.g. Speswhite™, English China Clay), bentonites, talcs, zeolites, calcium carbonate, and/or silicates.
- Binders, e.g. binders with a high melting point or indeterminately high melting points and of a non-waxy nature, e.g. polyvinyl pyrrolidone, dextrans, polyvinylalcohol, cellulose derivatives, for example hydroxypropyl cellulose, methyl cellulose or CMC. A suitable binder is a carbohydrate binder such as Glucidex 21D™ available from Roquette Freres, France.

- Waxes, such as organic compounds having a melting temperature of 25-150°C, preferably 35-80°C. Suitable waxes includes Poly ethylene glycols; polypropylenes or polyethylenes or mixtures thereof; Nonionic surfactants; Waxes from natural sources such as Carnauba wax, Candelilla wax, bees wax, hydrogenated plant oil or animal tallow; fatty acid alcohols; mono-glycerides and/or diglycerides; fatty acids and paraffines.
- Fibre materials such as pure or impure cellulose in fibrous form. This can be sawdust, pure fibrous cellulose, cotton, or other forms of pure or impure fibrous cellulose. Also, filter aids based on fibrous cellulose can be used. Several brands of cellulose in fibrous form are on the market, e.g. CEPO™ and ARBOCELL™. Pertinent examples of fibrous cellulose filter aids are Arbocel BFC200™ and Arbocel BC200™. Also synthetic fibres may be used as described in EP 304331 B1 and typical fibres may be made of polyethylene, polypropylene, polyester, especially nylon, polyvinylformate, poly(meth)acrylic compounds.
- Cross-linking agents such as enzyme-compatible surfactants, e.g. ethoxylated alcohols, especially ones with 10 to 80 ethoxy groups. These may both be found in the shell unit and in the enzyme core unit.
- Suspension agents, mediators (for boosting bleach action upon dissolution of the granule in eg a washing application) and and/or solvents may be incorporated as conventional granulating agents.



5 An important feature related to the smaller size of the core unit of the invention is that the volume, in which excipients are contained, is much smaller than the volume of known core units. Accordingly, for a calculated optimum concentration of an excipient in a core unit the absolute amount of excipient  
10 required to obtain this concentration is reduced. This feature reduces the manufacturing costs of a granule of the invention, because excipients often are expensive speciality chemical.

15 The shell unit of the invention is thicker than known shell unit and have a preferred thickness of at least 25  $\mu\text{m}$ . A more referred thickness is at least 50  $\mu\text{m}$  such as at least 75  $\mu\text{m}$ , at least 100  $\mu\text{m}$ , least 150  $\mu\text{m}$ , least 200  $\mu\text{m}$ , least 250  $\mu\text{m}$  or most preferably at least 300  $\mu\text{m}$ .

The components comprised in the shell unit composition may be selected from the list of excipient described, *supra*, in the 35 "enzyme core unit" section. Further components may be selected

35 "enzyme core unit" section. Further components may be selected

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in certain embodiments, act as a moisture and/or bleach barrier to stabilise the enzyme activity in the core unit. Furthermore, in alternative embodiments, the shell unit acts as a mechanical barrier during mechanical processes such as dosing or  
5 tabletting. In certain embodiments, the shell unit is sufficiently compressible and flexible for the enzyme core unit to withstand a tabletting process, both in a structural sense and with regards to activity. This is potentially most applicable for detergent formulations.

10 The shell unit, in many ways, resembles conventional shell unit or coating layers surrounding an enzyme containing core, except for the notable difference that it is thicker, preferably considerably thicker than known shell units. Also as opposed to conventional thin shell units, the shell unit of the  
15 invention contains very little enzyme. During preparation of an enzyme granule some of the enzyme in a core unit often undesirably passes or diffuses into the shell unit and may even reach the outer surface of the granule. However, in the present invention the increased thickness of the shell unit reduces the  
20 relative amount of enzyme in the shell, so that the amount of enzyme per weight of shell may be kept very low. Also by increasing the shell thickness the enzyme may be prevented from reaching the outer surface of the granule. Thus, the shell unit may be considered substantially free of enzymes in accordance  
25 with the definition used herein. The increased shell thickness of the invention reduces the amount of enzyme dust which may be released when handling the granules in a dry form, eg. as determined in the well known Heubach test method.

The shell unit provides protection to the enzyme in the core  
30 unit, because it physically separates the environment of the core unit, in which the enzyme is usually stabilised, from the environment surrounding the granule, which is usually hostile to the enzyme. Conventional thin shell units provides less protection, and it is necessary to incorporate expensive enzyme  
35 protecting agents in the shell unit, which neutralise harmful

components, which penetrate from the surrounding environment through the shell unit and into the core unit. By applying a thick shell unit this process is reduced, eg. by the distance between the core unit and the surrounding environment. In a preferred embodiment addition of enzyme protecting agents to the shell unit becomes obsolete and the shell unit is substantially free of enzyme protecting agents. By using the term "substantially free" in this context it is meant that enzyme protecting agents is not intentionally added to the shell unit. However, enzyme protecting agents from the core unit may during preparation of a granule pass or diffuse from the core unit into the shell unit. Accordingly, the term means that the concentration of enzyme protecting agent in the shell unit is less than 10% w/w the concentration in the core unit. The shell unit will also protect the enzyme in the core unit, when products containing granules of the invention is processed, such as steam-pelletising of feeds. The high temperatures used in the steam process can, under certain conditions, denature the enzymes thus reducing or destroying their activity. The shell unit may comprise components that confer thermal-resistance to the shell unit or whose overall composition gives a shell unit that will melt at a temperature at which the enzyme is still fully stable. This will allow the temperature within the immediate environment of the enzyme to rise no higher than the melting point of the shell unit for a certain period of time (the time in question is also dependent on the thickness of the shell unit). Accordingly a shell unit suitable for protecting an enzyme in the core unit during a (steam) pelletising process should have a melting temperature or temperature range within 70-120°C.

An important feature of the shell unit of the invention is that the increased thickness and composition of the shell unit contributes to granule properties such as, the overall activity, size and density of the granule. Accordingly in the present invention the activity of the final granule, the size

In a preferred embodiment the shell has an outer layer of  
25 a liquid lubricant. The purpose of the lubricant is to grease  
the granule so as to increase flow ability of the granule and  
to further inhibit dust formation when individual granules  
collide during handling. The lubricant is preferably a mineral  
oil or a nonionic surfactant, and more preferably the lubricant  
30 is not miscible with the other shell materials.

In ongoing research aimed at improving enzyme granulate formulations, with regards not only to granule properties, but  
35 also equally to process design and economy of design, a

conceptually new preparation process for preparing small core units of high enzyme activity surrounded by a thick shell unit has been developed. Accordingly the invention relates to a process for preparing an enzyme containing granule having a core-shell configuration, comprising the step of coating an enzyme containing core with a shell, so that the ratio between the diameter of the granule and the diameter of the core unit is at least 1.1.

In this process the preparation of a core unit may be physically separated in time and location from the process of coating the, preferably substantially enzyme free shell unit on the core unit and properties of the resulting granule may be adjusted and customised to specific applications by variation in the shell thickness and composition and by preparing core units having a narrow particle size distribution and a homogenous levels of enzyme.

When preparing enzyme granules of desired properties, a process wherein core units, which have a high or concentrated enzyme activity, are coated with a shell of an increased thickness offers several advantages:

- The core unit may be prepared independently of the process of applying the shell onto the core unit, because properties such as size, activity, density, colour, enzyme dust levels, odour, mechanical and physical strength etc. of the finished granule may be adjusted by the shell unit. This means that a wide range of different granules useful for different purposes and applications may be prepared using only few basic types of core units. The process of the invention provides huge logistic advantages because the core units may be prepared independently from the coating process, and may be stably transported and stored t suitable conditions as independent physical entities or product intermediates, which upon desire may be enclosed in a coating or shelling process to produce finished granule designed for a specific

- application. Storage conditions would preferably be where humidity levels and temperature are controllable or, where the enzyme cores are packaged, can be stabilised for e.g. in an airtight container. In fact there may be big differences in time and location between preparation of the core units and preparation of finished granules. The time difference or time span between preparing the core units and applying the shell unit for finishing the granules may be hours (1-24 hours), days (1-7 days), weeks (1-52 weeks) and even years (1-5 years), and the process provide for preparation of the core units in one geographical area (e.g. one country) and finishing granule in another geographical area (e.g. another country). Accordingly the storage stable core units may easily be shipped at low costs to a local finishing site for application of a shell unit which meets the specific needs of the intended local market. Also the concentrated enzyme core units provide reduced storage requirements, and reduced environmental risk in the packaging, shipping and handling.
- As many of the properties of the finished granule are conferred to the granule in the coating or shelling process methods for preparing core units may be chosen or developed which provides a narrow particle size distribution of the core units. Accordingly the process provides for reduced loss of enzyme activity during preparation, because the need further processing of the core units such as sieving, separating and re-circulating of over and under sized cores is reduced. Recycling processes are costly and incurs loss of active enzyme.
  - Energy savings are obtained by reducing recycling.
  - Production capacity is increased with decreasing recycle ratios.

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- Improved homogeneity in the finished granule activity.

The enzyme cores of the invention may be produced using techniques known *per se* in the art. Non-limiting examples of suitable techniques are spray cooling, spray drying, melt granulation and high shear granulation. A combination of more than one of these techniques may also be employed.

25 In a spray cooling process solidification of the mixture of enzyme in melted wax is achieved by atomising the mixture into droplets and solidifying the droplets in a stream of cooling air, typically in a cooling tower, whereby enzyme core unit particles having a narrow PSD can be obtained.

30           The enzyme may be applied to the molten wax by mixing a preferably purified crystalline or amorphous enzyme (such as described in WO 91/09943) into the molten wax. In a more preferred embodiment the enzyme and optionally other components are in a dry powder form such as spray dried products, which is  
35 dispersed or suspended in the molten wax. Atomization of the



A preferred special atomiser is a Rayleigh atomiser with which a particularly narrow particle size distribution may be obtained. One such atomiser is described in WO 94/21383. This  
35 atomiser allows for a process in which the amount of core units

that must be reprocessed due to being odd sized is considerably lowered. Although a spray cooling process is a very energy efficient process in that the heat of melting is much smaller than the heat of evaporation, it is not desirable to have any significant recycling of product due to capacity limitations and the risk of possible loss of enzymatic activity.

As an alternative core units may also be prepared by a process comprising making a dispersion of enzyme and optionally other components in a molten wax, letting the wax solidify and milling/crushing the solidified wax incorporating the enzyme particles and optionally rounding the particles, e.g. in a marumerizer process.

Another preferred alternative of preparing a wax based core unit (i.e enzyme containing particle) is a process comprising

- (a) dispersing or dissolving an enzyme in a molten wax,
- (b) transferring the dispersion to a liquid phase, e.g. an oil, in which both the enzyme and the wax are immiscible,
- (c) forming an emulsion of small droplets of enzyme-wax dispersion in the liquid phase,
- (d) cooling the liquid phase and the enzyme-wax droplets to solidify the wax into particles,
- (e) isolating the particles from the liquid phase.

For the purpose of the invention this type of process is denoted an emulsion granulation process.

Another possible embodiment to produce the enzyme core unit is a special spray drying process using the same or similar type of atomiser as the Spray cooling process, preferably the Rayleigh atomiser. This only requires a spray drying tower sufficiently large to allow the relatively large droplets to dry to the desired enzyme core size. This process route will result in a very efficient process; both with regards to energy and monetary investment.

In another embodiment of the invention, the enzyme core unit is produced by a melt granulation process. Melt granulation processes are known to the person skilled in the art (see *Melt agglomeration with polyethylene glycols in high shear mixers*, Torben Schæfer, The Royal Danish School of Pharmacy, 1996). It is may be preferred to add melt binder to the enzyme process prior to spray drying.

The enzyme core may be produced by a high shear granulation process in which the spray dried enzyme powder as produced by any of the preceding methods is mixed with components such as cellulose, dextrans, and sulfates before being transferred to a high shear mixer. A binder solution and sugar may be added in water until a desired mean particle size is achieved.

The enzyme core units can either be utilised directly after the preparation or they may be stored as an intermediate product, which can be processed later at the same production site or shipped to other specialised production sites, where several different products may be produced from the same enzyme core. This process is consequently very flexible compared to prior art, where only one product type might be produced at one time. In addition, the minimum feasible batch size is much smaller in the enzyme core process due to the small product hold-ups in the process. In one embodiment of the process, a thin film is applied to the enzyme core unit prior to shipping, storage, or immediate further processing to the final granule. The film layer can in certain embodiments aid in the subsequent shell coating step by comprising materials aiding in adhesion.

#### Application of shell units

Formation and application of the shell unit may also be performed using techniques known *per se* in the art, e.g. a mechanical coating process and/or a fluid bed coating process.

The coating step, i.e. addition of the shell to the enzyme core may be done as a pure mechanical coating process,

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A mechanical coating process may also be combined with a fluid bed drying step to enhance the production rate.

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### Using enzyme core as baking additive

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Certain maltogenic amylases can be used for prolonging the

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## Detergent Compositions

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Examples of useful amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the variants with substitutions in one or more of

the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

Commercially available amylases are Duramyl™, Termamyl™, 5 Fungamyl™ and BAN™ (Novo Nordisk A/S), Rapidase™ and Purastar™ (from Genencor International Inc.).

Cellulases: Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases 10 from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g. the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO 89/09259.

15 Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, 20 EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novo Nordisk A/S), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao 25 Corporation).

Peroxidases/Oxidases: Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g. from *C. cinereus*, and variants thereof as those described in WO 30 93/24618, WO 95/10602, and WO 98/15257.

Commercially available peroxidases include Guardzyme™ (Novo Nordisk A/S).

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**EXAMPLES**

The invention is illustrated by the following unlimiting  
5 examples.

**Example 1:**

3 kg of Savinase® enzyme (a protease enzyme available from Novo  
Nordisk A/S - Denmark) concentrate with a solids content of 33  
10 % w/w was added 10 % w/w of a dextrin binder. The enzymatic  
activity was approximately 98 KNPU/g in this mixture. The  
mixture was spray dried in a MobileMinor lab spray dryer using  
an 175 °C inlet air temperature, a 60 °C outlet air temperature  
and co-current atomization by a two-fluid nozzle to obtain a  
15 powder with an average particle size of about 20 µm. The  
obtained powder had an enzymatic activity of approximating 264  
KNPU/g.

The obtained powder is dispersed into 1 kg of melted PEG4000 at  
20 a temperature of 58 to 60 °C. The dispersion is spray cooled by  
atomising it in a spray cooling tower using a high speed  
rotational atomiser running at 9000 RPM. The obtained core  
units is screened to separate the fraction between 200 to 225  
µm.

**25 Example 2:**

Example 1 was repeated except that the a high speed rotational  
atomiser was replaced with a Rayleigh atomiser as disclosed in  
WO 94/21383, example 1, page 19, lines 12-36 in the spray  
cooling step. Also the Savinase® was replaced with a protein  
30 mixture (soy protein) and the PEG 4000 was replaced with a  
Lutensol AT-80 wax. The protein load was 40 wt %. Upon measur-

ing the obtained particle size distribution by a Malvern.laser instrument the following result were obtained:

| Property/RPM atomizer | 3300 | 4300 |
|-----------------------|------|------|
| D10, $\mu\text{m}$    | 206  | 199  |
| D50, $\mu\text{m}$    | 320  | 273  |
| D90, $\mu\text{m}$    | 464  | 387  |
| Span                  | 0.81 | 0.69 |

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Using a screen analysis the equivalent data on the same samples the following results were obtained:

| Property/RPM atomizer | 3300 | 4300 |
|-----------------------|------|------|
| D10, $\mu\text{m}$    | 203  | 185  |
| D50, $\mu\text{m}$    | 306  | 266  |
| D90, $\mu\text{m}$    | 397  | 350  |
| Span                  | 0.63 | 0.62 |

10 It is clearly seen from the above data that a very narrow size distribution is obtained even with the high protein load. The desired mean particle size may simply be obtained by adjusting the rotational speed of the atomizer. This enables a simple way of obtaining enzyme cores of a desired size and also having  
15 a narrow PSD and enables controlling the final enzyme activity of the finished enzyme granule comprising the core and the shell.

20 **Example 3:**

Example 1 was repeated except for the spray cooling step, which was replaced by a melt granulation process and the enzyme powder was replaced with a commercially available spray dried

soy protein powder (Soy-Co-Mill). 350 g of this powder was mixed with 95 g PEG 4000 chips and was added to a vertical high shear mixer (Mi-Mi-Pro from Pro-c-ept NV, Belgium). The powder temperature was raised to 66 °C using 1500 rpm impeller speed and 5600 rpm chopper speed. The obtained enzyme core particles was very compact and spherical, which greatly improves the later coating steps where the shell is supplied. A small amount of not agglomerated powder may stick to the surface of the particles if they are allowed to solidify in a non-moving system. Consequently, it will be preferred industrially to use a fluid bed cooler to solidify and to classify the obtained enzyme cores units.

#### Example 4

Example 3 was repeated with the exception that the soy protein powder was replaced by a spray dried enzyme powder made as in example 1, wherein the dextrin binder was added to the enzyme concentrate before spray drying is replaced by PEG 4000. This gave an efficient way of distributing a melt binder into the a spray dried powder before the melting and mixing process.

#### Example 5

Example 1 was repeated except for the spray cooling step which was replaced by a high shear granulation process in which the spray dried enzyme powder was mixed with 10% w/w cellulose fibres, 10% w/w dextrin binder. Sodium sulfate salt was added up to 1005 w/w. This mixture was transferred to a horizontal 50 L high shear mixer and mixed at under addition of a binder solution of having 5 % w/w dextrin and 5 % w/w sugar in water solution until a mean particle size of about 200 µm was achieved. The wet core units was subsequently dried in a fluid bed using 90 °C inlet temperature until the product temperature reached 60 °C. The dried product is screened to obtain core

units in the range from 180  $\mu\text{m}$  to 250  $\mu\text{m}$ . The resulting enzymatic activity was be approximately 14 KNPU/g.

#### Example 6

5 Example 1 was repeated except for the spray cooling step which was replaced by a emulsion granulation process wherein 100 g of the spray dried powder was dispersed into 100 g of a melted PEG4000 wax. This dispersion was subsequently emulsified using an Ultra Turrax blender into about one litre of mineral oil  
10 (Whiteway T15) heated to 65 °C. The size of the formed droplets of enzyme-wax dispersion in oil was controlled by the speed of the blender and the addition of an emulsifying agent (SPAN 80 which is a Sorbitan mono-9-octadecenoate (Monoester of oleic acid and hexitol anhydrides derived from sorbitol). When the  
15 desired droplet size was achieved the oil was cooled to ambient temperature, and the solidified particle were filtered from the oil. The formed core unit was calculated to about 132 KNPU/g. The obtained enzyme core particles was very compact, had a narrow PSD and a large number of these was perfect spheres,  
20 which greatly improves the later coating steps where the shell is supplied. This is due to the long time available for the surface tension to form the shape of the particle and the very low shear excerpted on the droplet during solidification.

#### Example 7:

25 A charge of 8 kg enzyme core produced as described in Example 5 was added to a fluid bed coater (Aeoromatic-Fielder Precision Coater. Size MP 2/3) to study the feasibility of such a fluid bed process for the initial coating of enzyme core. The aim of this study is to test the feasibility of the process to coat  
30 such a small enzyme cores without any agglomeration.

The initial properties of enzyme core were:

| Property | value |
|----------|-------|
|----------|-------|



|                    |                   |
|--------------------|-------------------|
| D10                | 185 $\mu\text{m}$ |
| D50                | 218 $\mu\text{m}$ |
| D90                | 251 $\mu\text{m}$ |
| Span               | 0.30              |
| Bulk density       | 0,797 g/ml        |
| Tapped density     | 1,10 g/ml         |
| Particle density   | 2,22 g/ml         |
| Savinase® activity | 14,32 KNPu/g      |

In the process following coating layers are applied:

| Coating layers                     | Amount applied |
|------------------------------------|----------------|
| additional enzyme layer            | 2537 g         |
| 2. layer : sodium sulphate + water | 7850 g         |
| 3. layer: HPMC + PEG400 + water    | 1600 g         |
| 4. layer : PEG 4000 + water        | 250 g          |

The processing conditions applied: 125 °C inlet air temperature  
5 and 50 °C product temperature.

The final properties of the coated enzyme core were:

| Property          | value             |
|-------------------|-------------------|
| D10               | 190 $\mu\text{m}$ |
| D50               | 241 $\mu\text{m}$ |
| D90               | 291 $\mu\text{m}$ |
| Span              | 0.42              |
| Bulk density      | 1,00 g/ml         |
| Tapped density    | 1,05 g/ml         |
| Particle density  | 1,82 g/ml         |
| Savinase activity | 15,00             |

The tapped density is measured by tapping a known mass of powder in a rigid container a specified number of times (typically 100 - 1000 times) and measuring the final volume of the powder. The tapped density is the ratio of the volume to the mass. The tapping is done by letting the powder container freely fall a specified distance (1 -10 mm) on a hard surface. HPMC is Hydroxy-propyl-methyl-cellulose.

The results shows that it is surprisingly possible to coat such small core units without the core units agglomerating in the process.

**Example 8:**

In this example the enzyme cores was produced by spray drying directly from a liquid concentrate using a Rayleigh atomiser as disclosed in WO 94/21383, example 1, page 19, lines 12-36. The liquid concentrates was formulated to achieve desired properties such as strength, viscosity and drying properties.

Following formulations was used:

- Test 1: 2500 l enzyme concentrate A, 1750 kg calciumcarbonate, 750 kg sugar and 400 kg water.
- Test 2: 2000 l enzyme concentrate B, 1500 kg calciumcarbonate, 91 kg sugar and 49 kg water.
- Test 3: 1400 l enzyme concentrate C, 350 kg calciumcarbonate, 91 kg sugar and 49 kg water.

Using a screen analysis on the obtained enzyme cores to measure the PSD the following results were obtained:

| Property/RPM atom-izer | Test 1: 4000 RPM | Test 2: 4000 RPM | Test 3: 4000 |
|------------------------|------------------|------------------|--------------|
| D10, $\mu\text{m}$     | 99               | 98               | 74           |
| D50, $\mu\text{m}$     | 192              | 192              | 201          |
| D90, $\mu\text{m}$     | 321              | 400              | 283          |

|      |      |      |      |
|------|------|------|------|
| Span | 1,16 | 1,58 | 1,04 |
|------|------|------|------|

These results shows the distribution of the unscreened product obtained directly from the spray drying including the fines fraction from the filter.

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Upon measuring the particle densities and enzymatic strengths of the spray dried enzyme cores the following results were obtained:

| Test / Property | Particle density g/ml | KNPU(S)/g |
|-----------------|-----------------------|-----------|
| Test 1          | 1,971                 | 21,7      |
| Test 2          | 1,780                 | 37,2      |
| Test 3          | 1,360                 | 122       |

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The produced particles was essential spherical and compact. The latter is seen from the true density data in Table 4. Current enzyme granulate from a high shear granulation process and having a comparable enzymatic strength has a true density which is very close to 1,9 g/ml. These results also show that using the Rayleigh atomiser it is possible in a stray drying process to produce strong non-agglomerated particles having a low size and a narrow PSD, which may suitable be used e.g. as dough improver.

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